The Effects of Hypoxia on *Hemigrapsus Oregonensis*

Introduction

The ocean is warming due to rising temperatures in the atmosphere creating a variety of physiological issues for marine life. According to Henry's law, as the temperature of water increases, it holds less gas which means that oxygen concentrations will decrease in the surface ocean. Ocean oxygen levels are also threatened by runoff containing excess nutrients which can cause algal blooms, eutrophication, and later, dead zones where oxygen is barely present at all. These conditions are what you call hypoxic. Natural ocean conditions such as strong stratification in the water column and upwelling of low O2, high CO2, and an abundance of nutrients can cause hypoxic areas to occur as well.

Hypoxic water is dangerous for marine life because too little oxygen for too long can result in mortality. Marine organisms that are at high risk include those that are stationary or move very little such as crabs, as opposed to a highly mobile organism like fish. When exposed to hypoxic conditions for a long time, most crabs die, but some of those that survive are briefly rendered immobile (Gravinese, 2020). In shallow tidal areas, crabs exposed to hypoxic water will spend more time on land than normal, allowing them to survive (Lima, 2015).

As hypoxic events are occurring more frequently, the fate of crabs is unknown. Therefore, this research seeks to understand how crabs will respond to hypoxia when they are in deep versus shallow water. This study will be performed using green shore crabs (*Hemigrapsus oregonensis*), which are native to the Puget Sound. The knowledge obtained from this study will be used to inform local crab fisheries and help predict the future of the crabbing industry.

Methods

Two plastic tanks were filled three quarters of the way with saltwater and nine hairy shore crabs (*Hemigrapsus oregonensis*) were placed in each tank. One tank was labeled "deepwater" and the other "intertidal". The air at the top of the deepwater tank was vacuumed out and the lid was sealed with tape. The intertidal tank contained a mesh wire ladder that extended into the exposed air, allowing the crabs to surface. The air was not vacuumed in the intertidal tank and the lid was not sealed shut. Both tanks did not have an air stone, and the saltwater had a salinity of 35ppt and 7-8 ppm of oxygen to start with.

The crabs were left alone for one week before the data was collected. After one week, 4 crabs were chosen from each of the tanks to run tests on. First, crabs were placed upside down in a third tank filled with freshwater and timed to see how long it took them to flip over. The crabs tested from the deepwater tank were labeled 1-4 with pink nail polish, and those tested from the intertidal tank were labeled 5-8.

Next, resazurin tests were conducted. 20mL of resazurin stock solution was made by adding 1g resazurin salt, 20mL DI water, and 20 µL DMSO. To prepare 300mL of working

resazurin solution, 296 mL seawater (DI water with Instant Ocean adjusted to 23-25 ppt), 666 µL resazurin stock solution, 300 µL DMSO, and 3 mL antibiotic solution 100x Penn/Strep & 100x Fungizone were combined. The 300mL working resazurin solution was divided into 8 crab chambers (35mL per chamber). Crabs 1-8 were weighed to the nearest hundredths of a gram and placed into a chamber which was then quickly covered with tin foil. Every 30 minutes, 200 µL of the solution was withdrawn from each chamber and placed in the wells of a 96 well culture plate. This was done 3 times over the course of 90 minutes. The plate was run through a spectrophotometer plate reader (Agilent BioTek Synergy HTX with software version 5) at excitation 530 and emission 590 to obtain fluorescence values. Each fluorescence value was then divided by its corresponding crab weight to normalize for differences in crab size.

All crabs were put back into their original containers and the DO for each was measured using a probe. The air in the deepwater tank was re-vacuumed and the lid sealed shut with duct tape. After another week, the righting and resazurin tests were conducted again. Hemolymph was extracted from the front claw of all living crabs, the crabs were euthanized and then dissected to look at gill tissue. DO was measured in each tank as well.

To measure lactate levels, hemolymph was extracted from 8 crabs. On a 96-well plate, 100 µL of Assay Buffer (1X), 20 µL of cofactor mixture, and 20 µL of the lactate substrate were added to all wells being used. 40 µL of enzyme mixture was added to the wells to initiate the reaction. The plate was covered and incubated at room temperature for 20 minutes and then the fluorescence was read using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. The average fluorescence of each standard and sample was determined and the corrected signal calculated. The corrected signal values of each standard were plotted as a function of the final concentration of L-Lactate to calculate L-Lactate.

Gill tissue states were categorized as “good” (gills were yellow/orange), “bad” (gills were dark/grey), “partial”, “pink” (from resazurin dye), or “undetermined”.

Results

After two weeks, all crabs from the deepwater treatment were deceased, and so was one from the intertidal treatment. This meant that there was no data for the deepwater treatment at the two-week mark. When comparing the righting time for both weeks, there was no significant difference in righting time between the two treatments. The resazurin results revealed that after one week, both hypoxia treatments had similar respiration rates that were higher than the control. After two weeks, the respiration rates for the intertidal treatment had dropped significantly but were still higher than the control. The hemolymph from six intertidal crabs had a range of lactate levels that were lower than the control. Two intertidal crabs had lactate levels around 250 µM, while the other four had lactate levels at or below 50 µM, compared to control which ranged from 300-420 µM. The gill tissue from the deepwater crabs was 89% bad and 11% partial. The gill tissue from the intertidal crabs was 44% bad, 33% partial, 11% pink, and 11% unusable. The gill tissue from 2 control crabs were both categorized as good and used as a reference.

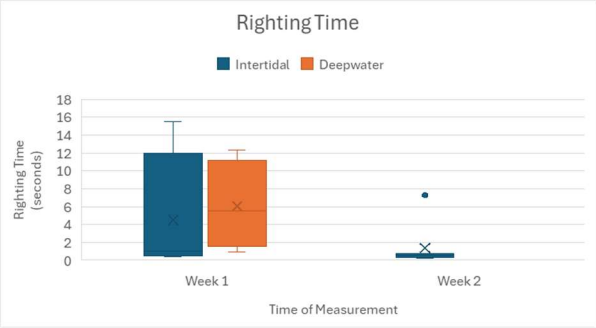

Figure 1: Righting time for both intertidal and deepwater crabs.

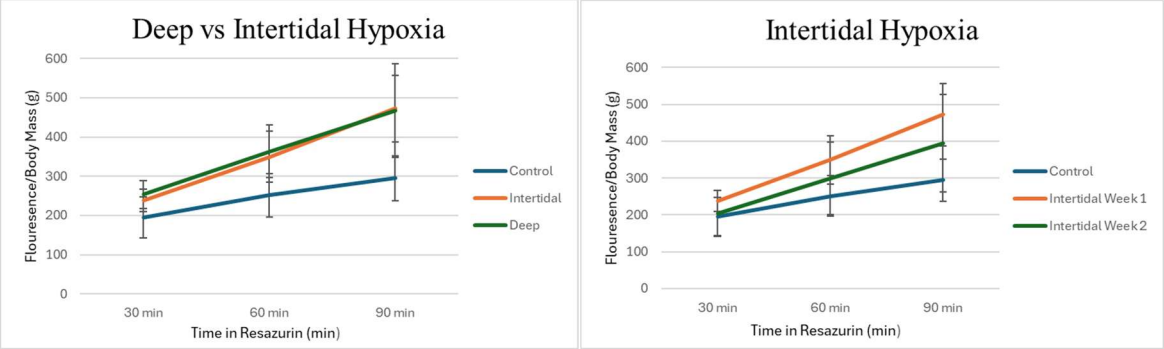

Figure 2: Resazurin results after 1 week in hypoxic conditions.

Figure 3: Resazurin results for the intertidal treatment after 2 weeks in hypoxic conditions.

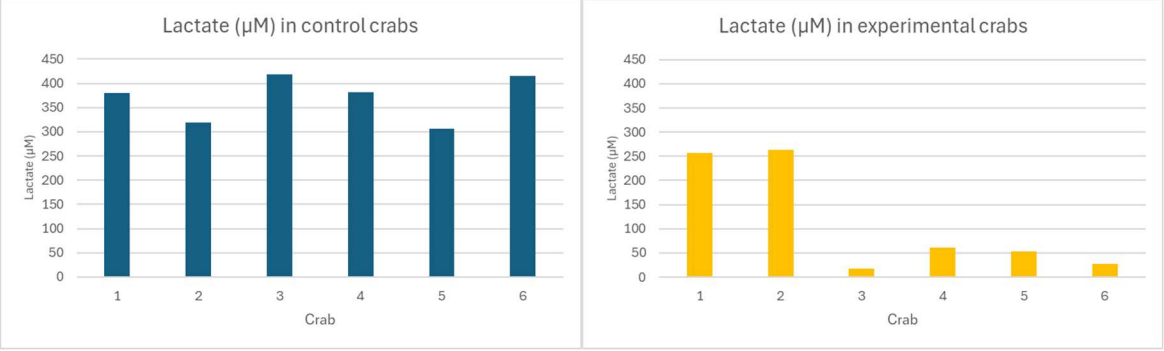

Figure 4 and 5: Lactate levels found in the hemolymph of intertidal crabs

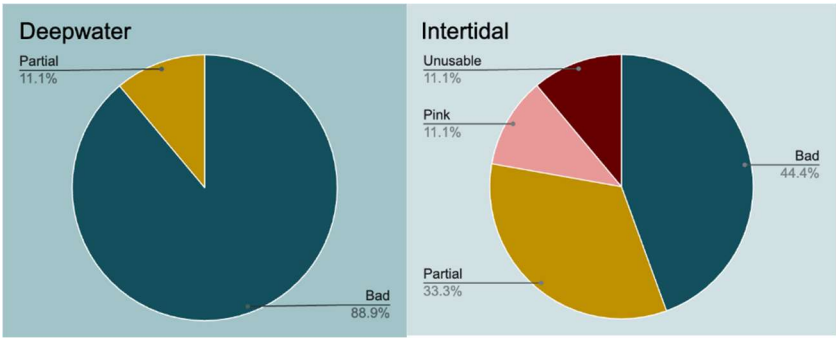

Figure 6 and 7: Gill tissue diagnosis for deepwater and intertidal crabs.

Figure 8 through 11: Gill tissue conditions from left to right; good, partial, bad, and pink.

Discussion

The most definitive result of this study is that all the crabs from the deepwater treatment died after a week. This is consistent with other studies when exposing crabs to severe hypoxic events. In some cases of extreme hypoxia, crabs with no access to air started to die after 10 hours (Lima, 2015).

When considering righting time in both treatments, we expected righting time to increase over the 2-week span assuming crabs would be slower to flip themselves around. This is because crabs have demonstrated increased lethargy in response to intense hypoxia in previous studies (Gravinese et al, 2020). However, this was not the case. Crabs from either treatment showed no difference in righting time, demonstrating that this is not a strong determining factor of physiological stress.

According to McGaw (2008), having a higher heart rate increases blood flow and thus the delivery of oxygen to the body which may explain why respiration rates increased in both the deepwater and intertidal treatment. Yet, respiration rates dropped in the second week which may have been a result of long-term stress.

Contradicting to the expected lactate levels, the crabs from the intertidal treatment had significantly lower lactate in their hemolymph than the control crabs. We expected this to be the opposite because when oxygen concentrations in the water drop below critical levels, crustaceans cannot use aerobic metabolism anymore. They must redirect blood flow to supply the most important tissues with oxygen by switching to anaerobic metabolism. As a result, lactate would increase in the hemolymph, and instances of hypoglycemia would occur more frequently (Geihs et al, 2013). However, the experiments' results can be explained by the "lactate paradox". When organisms get accustomed to low levels of oxygen, their production of lactate will drop below normal levels (West, 2007).

The deterioration of the gill tissue was much worse in the deepwater treatment than in the intertidal treatment. The dark gill tissue had atrophied and started to decay as a result of low oxygen levels in the water. For crabs that are experiencing hypoxic conditions in the wild, one would expect their gill tissue to start to decay as well. However, it is unknown whether the gill tissue will regenerate after the hypoxic event is over. While there is some variation in lethargy

and lactate levels between previous studies and our own, increased respiration rates during hypoxia seem to be a consistent result. These results may hold true for other species of crabs. If scientists and commercial crabbers expect these physiological effects to take place in their native crab species during hypoxic events, it may help them be able to protect them better.

Hypoxic events caused by natural and anthropogenic factors are becoming more frequent and intense. However, if the crabs at risk have access to air, there is a much higher chance that they will survive. This might be the key to keeping the crabbing industry alive.

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